4.0 mM in the experiments illustrated in Fig. 1, 0.5 mM in those illustrated in Fig. 2.

In studies designed to test this action of EGTA, an increase in EGTA from 0.5 to 4 mM in the presence of Mg at a concentration greater than 1 mM caused nearly complete relaxation. The inhibition of contraction by concentrations of Mg greater than 0.1 mM is completely reversed by the addition of $10^{-5}M$ free Ca^{++}. Maruyama and Watanabe (*6*) have reported similar biphasic effects of Mg on the superprecipitation and adenosine triphosphatase activity of myosin B from skeletal muscle, and recently the same has been reported for glycerinated psoas muscle (*7*).

Vascular smooth muscle does not develop tension unless the Mg concentration of the bath closely approximates the ATP concentration, even though free Ca^{++} is present. With 5 mM ATP and free Ca^{++} in the bath (that is, under conditions approximating those in active muscle), the Mg requirement of vascular smooth muscle is much greater (at least tenfold) than that of skeletal muscle. This contraction of glycerinated vascular smooth muscle in high Mg concentrations is not produced by release of chelated Ca since in these studies (Fig. 2, upper curve) the concentration of Ca^{++} was sufficient to give a maximum contraction.

In its dependence on Ca and Mg, glycerinated skeletal muscle behaves as one might predict from studies of adenosine triphosphatase activity and superprecipitation. Similar values for Ca^{++} concentrations for threshold and maximal activity have been found for the glycerinated muscle model, adenosine triphosphatase activity, and superprecipitation. The dual effect of Mg, activation or inhibition depending on its concentration, also is qualitatively and quantitatively similar in all three. Thus a substantial link is added in the chain of evidence suggesting that the properties of actomysin which are rate-limiting for its adenosine triphosphatase activity and superprecipitation are, indeed, the same as those that are rate-limiting, *in situ*, for the development of tension.

The glycerinated smooth muscle model exhibited a striking similarity to skeletal muscle in the Ca^{++} requirement for contraction. On the other hand, the Mg^{++} requirements of these two muscles were distinctly different. The high concentration of this cation required for contraction of smooth muscle raises the possibility that it may have a regulatory role in the contraction of this tissue.

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Noradrenaline Stores in Nerve Terminals of the Spleen: Changes during Hemorrhagic Shock

Abstract. *In dogs subjected to hemorrhagic shock, a marked decrease in the noradrenaline content of the sympathetic nerve terminals in the normally innervated spleen is revealed by means of a histochemical fluorescence method. Deprivation of the sympathetic impulse-flow to the tissue immediately before the animals are subjected to shock prevents this depletion. The results support the hypothesis that the vasoconstriction which occurs during shock is due to the effect of noradrenaline released locally in the tissues, and not to circulating noradrenaline.*

We have reported that the tissue content of noradrenaline markedly decreases during hemorrhagic and endotoxic shock in dogs and in rabbits (*1*). It has also been reported that sympathetic denervation or blockade of the sympathetic nerves protects the tissue from otherwise irreversible damage in shock (*2, 3*). It was suggested that the abundant release of noradrenaline from the sympathetic nerve terminals in the tissue during shock, rather than circulating catecholamine, causes vasoconstriction and impairment of blood flow and tissue oxygenation.

We have studied the noradrenaline content of the nerve terminals in the spleen by the fluorescence method of Falck and Hillarp (*4*) which is highly specific for monoamines in, for example, the cell bodies, axons, and terminals of adrenergic neurons. The tissue, taken from different parts of the spleen at different times, was freeze-dried, treated with formaldehyde gas, embedded in paraffin, sectioned, and mounted for fluorescence microscopy. By this treatment noradrenaline is converted to an intensely green fluorescent substance, easily recognized in the fluorescence microscope used.

Dogs weighing 20 to 25 kg were subjected to irreversible hemorrhagic shock by a standardized procedure (*1*) according to which the blood was collected in an open reservoir by means of a catheter inserted in a femoral artery. The aortic pressure was kept constant at 35 mm-Hg by adjusting the height of the reservoir. When the animals had taken back 40 percent of the maximum amount of blood removed they were given back the remaining amount of blood in the reservoir. With this technique most dogs die in irreversible shock 2 to 4 hours after transfusion. The spleen was examined by laparotomy. Either 4 weeks before or immediately before the dogs were subjected

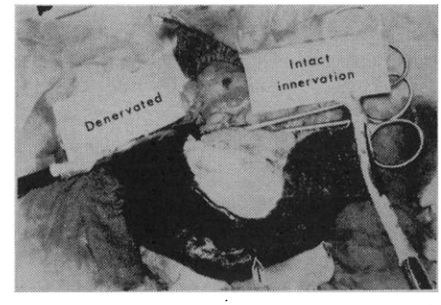

Fig. 1. The spleen of a dog bled for 3 hours. The contracted half of the spleen with intact innervation is on the right; on the left is the denervated half, unchanged in size. Before bleeding, these two parts were about the same size. The borderline between the two parts is indicated by the arrow.

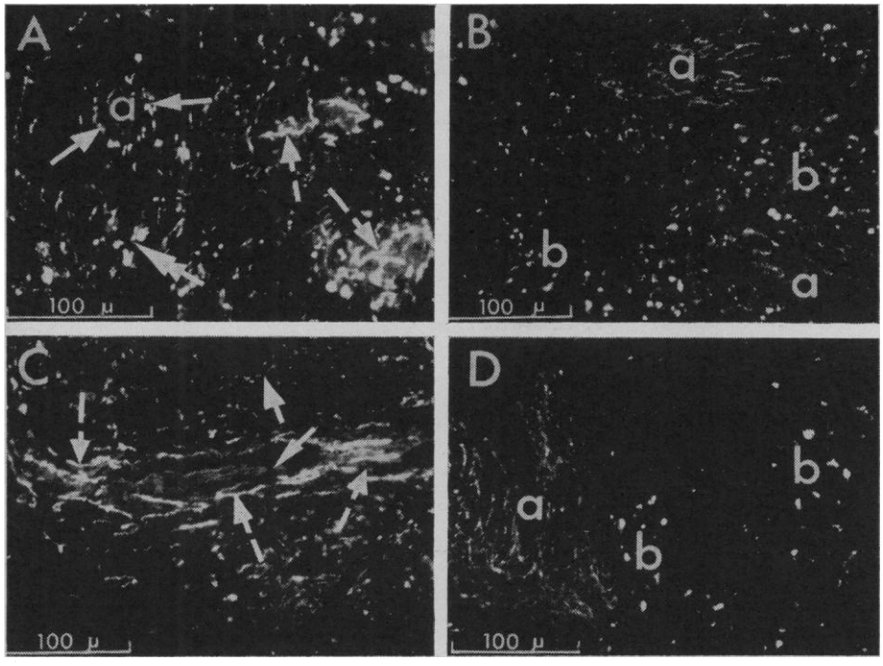

Fig. 2. (*A*) Normal spleen tissue. *a*, A transversely cut vessel with a rich supply of adrenergic terminal bundles (arrows). The fine, irregular, strongly green-fluorescent terminal bundles in tangentially cut parts of the trabecular network are indicated by broken arrows. Yellow autofluorescent products (double-headed arrow) are scattered throughout the tissue. (*B*) Spleen tissue with intact innervation at the end of the shock experiment. No adrenergic fibers can be seen; the tissue is contracted, and only smooth, coiled, elastic fibers of the trabecular network (*a*) exhibit their characteristic autofluorescence. *b*, Yellow autofluorescent particles. (*C*) Spleen tissue, deprived of its sympathetic innervation just before the dog was subjected to shock, at the end of the shock experiment. A longitudinally cut vessel with its unchanged, rich supply of strongly green-fluorescent adrenergic terminals is indicated by broken arrows. Other arrows point to elastic fibers. (*D*) Spleen tissue, denervated 4 weeks before the dog was bled. No adrenergic fibers can be seen. *a*, Elastic fibers in the trabecular network; *b*, yellow autofluorescent products.

to shock, half the spleen was denervated by sectioning the nerves adherent to one of the main arterial branches. During the experiment the spleen was kept in a plastic cover so that samples could be obtained and changes in size and appearance could be observed. Samples for examination by fluorescence microscopy were taken from both the denervated and the normally innervated part of the spleen before shock was induced and every 2 to 3 hours thereafter until death.

The normal spleen in dogs has a very rich supply of adrenergic fibers. With the fluorescence microscope, the terminals are revealed as rather fine, varicose, green-fluorescent fibers, running together in small bundles. They have the typical appearance of peripheral adrenergic terminals (*5*), situated both around the vessels in the white and red pulp and in the trabecular network (Fig. 2*A*). Sometimes single-terminal bundles are also scattered in the red pulp without any obvious relation to vessels or trabeculae. The terminals are often intermingled with elastic fibers, which have a green autofluorescence similar to the specific fluorescence of the terminals, but which are of a thin, smooth appearance, quite different from that of the adrenergic fibers. The spleen also contains autofluorescent products, many of them probably derived from the breakdown of red corpuscles. These products appear as irregular, yellow-fluorescent spots scattered throughout the tissue.

In the part of the spleen with intact nerves (Fig. 2*B*) the green noradrenaline fluorescence gradually disappeared during shock. Some terminals retained their fluorescence longer than others; 3 hours after the onset of bleeding a large number of adrenergic fibers had completely disappeared while some showed only a weak to medium fluorescence intensity, and still others were apparently unaffected, and showed a strong fluorescence intensity. Just before the dog died, the fluorescence had further decreased and was very weak to completely absent. During the hypovolemic period the normal part of the spleen was contracted (Fig. 1), but became engorged and distended with blood when normovolemia was restored. In the part where the nerves had been cut the amount of specific green fluorescence was the same at the end of the experiment as before (Fig. 2*C*), and no change was observed in the size of the spleen.

In the spleen denervated 4 weeks before the experiments, no specific fluorescence was apparent in samples obtained before shock was induced (Fig. 2*D*) and none appeared during the shock state. No change in size was observed in the denervated part at any time.

Our work demonstrates the presence of noradrenaline stores in the sympathetic nerve fibers and shows that the catecholamine content of these fibers is rapidly reduced during shock. In tissue deprived of its sympathetic innervation just before the animal is subjected to shock, the noradrenaline content does not change. This confirms the results obtained in a previous study (*1*) in which a chemical method (*6*) was used, and indicates that the release of noradrenaline is caused by the continuous flow of impulses in the sympathetic nerves.

The denervation of the spleen 4 weeks before the dogs were subjected to shock led to a complete depletion of the stores of noradrenaline. The absence of noradrenaline in the terminals did not seem to interfere with function; thus the type of denervation performed protects the tissue from damage as effectively as does transsection or blockade of the sympathetic nerves immediately prior to shock (*2*). These observations, and the fact that the size of the denervated part of the spleen does not change significantly (*3*), indicate that at least the main influence on the smooth muscles in the spleen is exerted by the locally released noradrenaline, rather than by the circulating amine.

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Inheritance of Linoleic and Oleic Acids in Maize

Abstract. *Gas-liquid chromatographic analysis of methyl esters of fatty acids of individual maize seeds of parental and segregating populations suggests that desaturation at the Δ^{12-13} position in oleic acid is under simple Mendelian control. High linoleic acid content is recessive to low.*

Biosynthesis of unsaturated fatty acids in higher plants remains essentially unexplained. According to Erwin and Bloch (*1*) a mechanism distinctly different from two relatively well characterized biosynthetic pathways (anaerobic and oxidative desaturation) appears to exist in higher plants. In the "plant pathway," neither stearate nor palmitate serves as a precursor of oleic acid, but myristate and laurate are transformed to oleate (*2*). Bloch further suggested that oleate is progressively desaturated toward the methyl end of the molecule that is producing linoleic and linolenic acids.

The possibility that the orderly creation of additional double bonds in the oleic acid molecule might be under genetic control presents an attractive problem to a geneticist, particularly one working with maize. Accordingly, an effort was made in the winter of 1963–64 to analyze genetically the distribution of C_{18} fatty acids in the F_1, F_2, and backcross progenies of two strains of maize, R84 and Illinois High Oil (IHO). The two were selected primarily because of their contrasting distributions of fatty acid: R84 was high in linoleic acid and low in oleic acid, whereas IHO was lower in linoleic and higher in oleic.

Gas-liquid chromatographic analyses were made on individual kernels of parental, F_1, and segregating populations. Individual kernels were crushed and the oil was extracted with petroleum ether. The oil was then esterified (*3*). The esters were dissolved in approximately 1 ml of petroleum ether and 3 μl of the solution was injected in the chromatograph for analysis. Some early difficulty was encountered in making satisfactory determinations with so small an amount of oil, but refinement of the extraction methods provided sufficiently large oil samples to make the method successful.

Reliability of the chromatographic analyses was examined by repeated sampling of single kernels. No significant differences were found among repeated individual runs on the oil from individual seeds. The standard error associated with individual-kernel measurements was 1.32 percent for linoleic acid and 0.78 percent for oleic acid.

All analyses were made on an Aerograph Hi-Fi Model 600 gas chromatograph with a 1.5-m, 0.15-cm column packed with diethyleneglycol succinate on acid-washed chromosorb W. The column temperature was maintained at 180°C. Helium flow was adjusted to 18 ml/min. A flame ionization detector was used.

An examination of the frequency distributions in backcross and F_2 populations (Figs. 1 and 2) suggests monohybrid inheritance for oleic and linoleic acid content. The data support the hypothesis that the low linoleic acid content is dominant to high, and the low oleic acid content is recessive to high.

Table 1. Means and ranges of linoleic and oleic acid content (percentage of total oil) of individual maize kernels of parents, F_1, F_2, and backcross generations.

Linoleic (%)			Oleic (%)	
Range	Mean		Range	Mean
		R84 ⊗		
57.0–65.5	61.3		21.5–28.8	24.5
		IHO ⊗		
40.5–53.9	48.8		30.5–43.4	35.3
		F_1 (*R84* ♀)		
47.0–55.6	52.2		21.3–35.1	31.3
		F_1 (*IHO* ♀)		
42.0–52.1	47.6		31.3–42.6	36.1
		BC R84		
46.2–61.0	54.3		22.6–38.2	29.1
		BC IHO		
45.5–58.7	51.2		25.3–38.9	32.5
		F_2		
40.9–63.2	51.5		20.8–46.0	32.3

Chi-square analyses of pooled F_2 linoleic acid data indicate a poor fit to a 3:1 ratio. However, analyses of individual F_2 ears of the maize revealed that the ratio in only one ear out of six was quite deviate, and that the remaining five had chi-square probability values of 0.5–0.8. The backcross data were particularly convincing in that pooled data on the backcross of the F_1 to the high linoleic acid parent gave a bimodal distribution. Backcrosses of the F_1 to the low parent gave unimodal distributions.

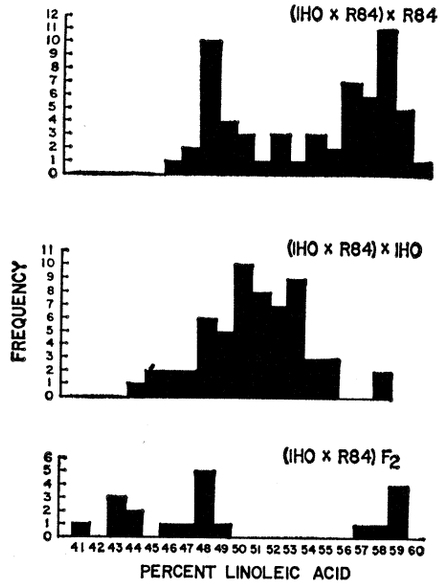

Fig. 1 (left). Frequency distributions of linoleic acid contents of individual maize kernels of backcross and F_2 generations.

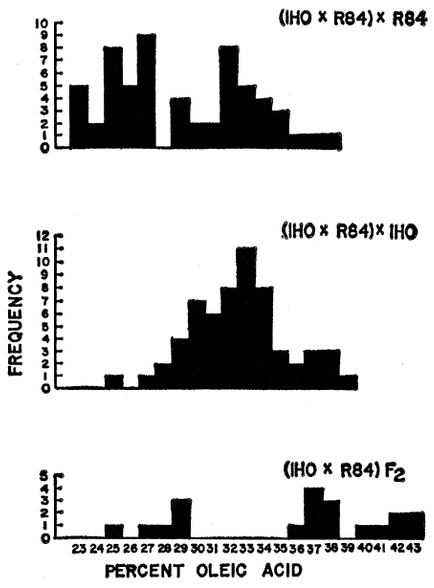

Fig. 2 (right). Frequency distributions of oleic acid contents of individual maize kernels of backcross and F_2 generations.